

## Electronic letter

### Fetal bowel hyperechogenicity may indicate mild atypical cystic fibrosis: a case associated with a complex *CFTR* allele

EDITOR—Cystic fibrosis (CF) is an autosomal recessive disorder affecting 1/2500 live births in northern European populations<sup>1</sup> and is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.<sup>2</sup> Data accumulated over the past 10 years have produced a remarkable repository of mutations and polymorphisms of the *CFTR* gene.<sup>3</sup> However, the clinical consequences of many rare mutations are still poorly understood and functional studies are not routinely available. Because of the mutational heterogeneity and the rarity of many mutations, most clinical DNA laboratories offer tests that aim to detect 85–90% of CF alleles, and the systematic, cumbersome analysis of the rest of the gene is performed in selected cases only. With the increasing demand for prenatal screening of pregnancies from the general population, an increasing number of CF patients are diagnosed in utero in the absence of a family history of CF, either after preconceptional genetic screening of the parents, or after a routine fetal ultrasound showed a CF associated finding, for example, fetal bowel hyperechogenicity (FBH). In such instances, reliable genotype-phenotype correlations are required for appropriate counselling. FBH is defined as an echogenicity of a fetal bowel loop similar to or greater than the fetal iliac crest<sup>4</sup> and is found in 0.5–1% of all second trimester pregnancies.<sup>5</sup> Whether hyperechogenicity in FBH originates from the bowel wall or from intraluminal content remains a matter of debate.<sup>4</sup> It is a non-specific sign that is associated with CF in some cases.<sup>1–4,6</sup> In a study of 209 pregnancies with FBH, 68% had a subsequent normal outcome and 3.3% had CF, the other cases corresponding to chromosomal abnormalities (5%), gastrointestinal malformations (8%), fetal infection (4%), or unexplained fetal death (13%).<sup>5</sup> Before 18 weeks' gestation, the assay of gastrointestinal enzymes in amniotic fluid may help in establishing a diagnosis, but the results become uninterpretable after 18 weeks.<sup>7</sup> Because of non-specificity of FBH and of incomplete sensitivity of routine *CFTR* mutation screening, FBH is often a challenge for genetic counselling, especially if found after 18 weeks' gestation. If only one *CFTR* mutation is found in a fetus with FBH who has a normal karyotype and no evidence of infection, this can correspond either to a normal heterozygous carrier of CF (4% of the white population) or to an affected compound heterozygote for a rare *CFTR* mutation undetected by routine DNA analysis. For appropriate genetic counselling, a systematic *CFTR* gene study should be performed in the apparently non-carrier parent and fetus, very quickly during the continuing pregnancy, and realistic predictions must be conveyed regarding the expected phenotype when a second mutation is found.

We report on a sibship of two brothers and an ongoing pregnancy, in all of whom two mutated *CFTR* alleles were identified after FBH ascertainment. The older brother was asymptomatic while the younger had some respiratory history and a slightly abnormal sweat test.

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A hyperechogenic fetal bowel was found on a routine ultrasound screen at 22 weeks' gestation in the third pregnancy of a 33 year old, gravida 3, para 2 Belgian mother of two healthy boys aged 6.8 and 2.6 years (fig 1). The abnormal bowel loop displayed the same echogenicity as the fetal iliac crest, and no ascites nor sign of ileus nor ectopic calcification were observed. The parents were not consanguineous and had unremarkable personal and family histories. Amniotic fluid was sampled for standard karyotyping, which was normal (46,XX), and microbiological studies, which showed no evidence of infection. Amniocyte DNA and peripheral blood DNA from both parents were analysed for CF mutations in a routine test aimed at 10 mutations that cover 85% of all mutated alleles in the Belgian population. The N1303K mutation was found in the fetus and the father and no mutation was found on the maternal allele or in the mother. The family was recalled to the Genetic Counselling Clinic. A history of two episodes of lower respiratory tract infection was reported in the youngest child, whose weight, height, and physical examination were within normal limits. The routine DNA analysis showed the presence of the paternal N1303K mutation in both the younger and the older brother. *CFTR* gene studies were pursued using a DGGE scanning strategy,<sup>8,9</sup> and the maternal *CFTR* allele was found to carry three missense mutations, D443Y (1459G>T, exon 9), G576A (1859G>C, exon 12), and R668C (2134C>T exon 13), which have each previously been reported in males with CBAVD.<sup>10–12</sup> On further analysis, the complex mutated maternal allele was found in the younger boy. Because of this finding, a sweat test was performed in him which showed a slightly increased chloride concentration (35 mEq/l, with a normal upper limit of 28 mEq/l in this age group). More surprisingly, compound heterozygosity was also found in the strictly asymptomatic older brother. The family was counselled for atypical CF with a mild clinical course, at least in childhood as observed in the two brothers, with the possibility of a probably mild CF related lung disease later in life in any of the children. A normal 3150 g baby girl was delivered at term. The parents declined sweat testing in their oldest son.

We were helped in this genetic counselling by the observation of the two healthy, or hardly symptomatic, brothers with the same genotype. FBH being found in 0.5–1% of all pregnancies, we face this difficult counselling situation on

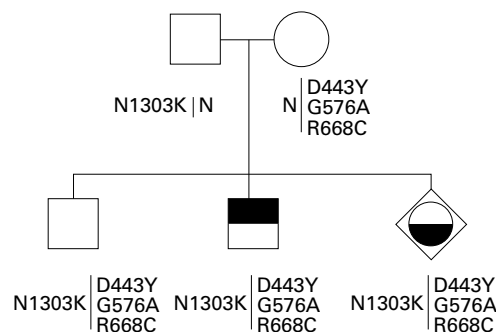


Figure 1 *CFTR* genotype and clinical findings in the family. The two brothers and fetus are compound heterozygotes for the N1303K mutation and the complex *CFTR* allele D443Y-G576A-R668C. Upper half filled symbol: history of lower respiratory tract infection and slightly raised sweat chloride. Lower half filled symbol: fetal bowel hyperechogenicity. No information is available regarding fetal bowel echogenicity in the brothers and a sweat test was not performed in the oldest.

a regular basis and propose the following approach. Genetic counselling is indicated in each case of FBH. In the absence of a known family history of CF, we recommend fetal karyotyping and serological and microbiological studies targeting micro-organisms that may cause FBH, as well as gastrointestinal enzyme assays in the amniotic fluid if FBH is observed before 18 weeks' gestation, as normal enzyme assays virtually rule out CF.<sup>7</sup> If enzyme assays are abnormally low, or if FBH is found after 18 weeks, we offer *CFTR* gene screening, which currently detects 85% of CF alleles in the Belgian population, in the parents and fetus. If both parents test negative, we consider CF unlikely, unless the parents belong to an ethnic subgroup where other specific mutations should be sought. If one or both parents test positive and the fetus tests negative, CF is virtually excluded. If a CF mutation is detected in one parent only and in the fetus, we perform a systematic analysis of the other allele using a DGGE sequencing strategy.<sup>8-9</sup> We check paternity whenever false paternity can significantly modify risk figures. When a second mutation is not found, the parents may be reassured although CF cannot be totally excluded. The residual risk is complex and must combine the FBH related risk and the risk based on mutation analysis, which takes into account the ethnic background of the parents. According to Hodge *et al.*,<sup>13</sup> and considering a 3% risk of CF in FBH, a 4% carrier frequency, and a 95% sensitivity of the DGGE approach when applied to the whole coding sequence, the residual risk of CF in a fetus bearing one detectable CF mutation inherited from one parent might be as high as 0.07.<sup>14</sup> Segregation studies of *CFTR* gene polymorphisms in sibs, if available, may, in theory, lead to CF exclusion if a healthy sib shares the same genotype with the fetus. In our case, however, the discovery of FBH led to an unexpected diagnosis of mild CF, at least in the younger boy.

D443Y, G576A, and R668C have been observed independently or in pairs, in patients with a CF related syndrome for whom the whole *CFTR* coding sequence has been analysed: D443Y, G576A, R668C, D443Y-G576A, D443Y-R668C in CBAVD patients<sup>10-12</sup> and G576A-R668C in a patient with disseminated bronchiectasis, but with no other CF causing mutation found in trans.<sup>15</sup> To our knowledge, the D443Y mutation was only observed in CBAVD patients.<sup>11-12</sup> The G576A and R668C variations have both initially been described as polymorphisms since they were found on the non-CF chromosome of the mother of a CF child.<sup>8</sup> However, they were later considered as putative mild mutations associated with a CBAVD phenotype when combined in trans with  $\Delta F508$ .<sup>16-17</sup> These genotypes may possibly not be disease causing in women.

We report here the first case where the three variations are associated. The fact that these mutations have been found independently as well as in different combinations is quite surprising. Either they result from frequent mutational events and should hence be associated with various haplotype backgrounds, or one might question the sensitivity of the methods used in previous reports. Assuming the mutations appeared one after another on the same ancestral chromosome, it is possible that the resulting allele was selected for greater loss of function conferring greater advantage to the heterozygous carrier. Indeed, mild *CFTR* mutations or variants combined in cis may produce a more deleterious effect than each mutation alone, as was recently shown by functional studies in transfected cells for the D1270N-R74W mutations,<sup>18</sup> confirming previous observations.<sup>19-20</sup> The identification of an increasing number of complex alleles<sup>3-21</sup> may partly account for the difficulties in establishing genotype-phenotype correlations. In another fetus with FBH at 21 weeks' gestation, we found the genotype W846X/G576A-R668C. The parents

were counselled towards CBAVD at worst and a healthy boy was born at term. Both the immunoreactive trypsin test and the sweat test gave normal results. It is thus possible that, in our family, the D443Y variation worsens a very mild deleterious effect of the G576A-R668C allele, or vice versa, accounting for the abnormal sweat test and, perhaps, the respiratory infections in the younger brother.

Importantly, this family indicates that FBH is not necessarily associated with a severe outcome in CF. Conversely, meconium ileus is usually associated with pancreatic insufficiency and a severe outcome.<sup>22</sup> Of note, meconium ileus did not develop in this pregnancy, and both of the brothers clinically appear to have pancreatic sufficiency. Although follow up in this baby girl should confirm pancreatic sufficiency, and although no data are available regarding bowel echogenicity during fetal life in the brothers, our observation favours the hypothesis that FBH is not causally related to pancreatic insufficiency or meconium ileus, and reflects an intrinsic defect of the intestine wall, perhaps inspissated secretions in the glandular crypts, rather than accumulation of viscous meconium in the gut.

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